Vet. Med. J., Giza. Vol. 59, No. 3 (2011): 435 - 445

Multidirectional fashion of immune response induced by fowl pox vaccine co-administered with inactivated AI-ND vaccine

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Received: 22/04/2011
Accepted: 02/05/2011

SUMMARY

In the present study, the immune stimulatory effect of poxvirus vaccine on the humoral and cellular immune response to AI-ND inactivated vaccine was assessed. Three groups of commercial broiler chicken were treated as follows: 1. group 1 was kept non-vaccinated as control birds. 2. Group 2 was vaccinated at 10 days old with inactivated AI-ND vaccine (H5N2) and co-injected S/C with fowl pox vaccine. 3. Group 3 was vaccinated at 10 days old with AI-ND vaccine. The chickens were challenged with VVNDV 4 weeks post vaccination to determine the protection induced in different groups.

Humoral and cellular immune response of the chickens were monitored on weekly base by different assays including nitric oxide, lysozyme activity, phagocytic activity, and HI. The results revealed 100% protection, significant increase in phagocytic activity of macrophage, increased serum lysozyme in chicken co-administered the fowl pox with AI-ND vaccine compared to those vaccinated only with AI-ND vaccine (80%) or the non vaccinated control birds (0%).

The results of the experiment clearly demonstrated the immune stimulatory effect of fowl pox vaccine on the immune response to AI-ND vaccine confirming the complex – antigen specific effect of poxvirus with differential synergy of such response based on the order of administration when given as primary. This study reports the useful use of poxvirus as primary live replicating virus and its stimulatory effect on the specific immune response of the boosting antigens in order with

widely divergent level of immunity. Also, the study emphasizes the importance of using poxvirus in the prime-boost concept.

INTRODUCTION

The immune system is molded by histories of infection, and prior exposure to one pathogen may influence resistance to a second unrelated pathogen. There are a phenomenon known as heterologus immunity, the immune memory lymphocyte repertoire created in response to one pathogen may influence the immune response to another unrelated pathogen (Welsh and Selin, 2002). Hosts acutely infected with many viruses can generate very high levels of type 1 interferon that provide strong resistance to super infection (Welsh, 1984). To confirm this previous concept, Barton et al., (2007) found that mice latently infected with murine cytomegalovirus or gammaherpes virus chronically reactivate these viruses, whose antigen stimulate IFN-γ production by memory T-cells; this response provides resistance to Listeria monocytogens and Yersinia pestis, which grow poorly in IFN-γ activated macrophages.

Santra et al., (2007) demonstrated that monkeys vaccinated with recombinant modified vaccinia virus Ankara (rMVA)/ recombinant fowl pox virus boost regimens developed comparable cellular immune responses that were greater in magnitude than those elicited by a homologous prime/ boost with (rMVA) recombinant monkey virus Ankara. Where as, a variety of poxviruses have been developed as potential vaccine vectors (Haig and Fleming, 1999, Haige et al., 2002), including vaccinia virus, modified vaccinia virus Ankara (MVA), NYVac, canarypox, and fowl pox (FPV). It may prove the possibility to combine disparate pox vectors to elicit robust cellular immune responses.

The aim of this study is to propose a vaccination scheme capable of inducing higher immune response as well as protection against the challenge velogenic NDV strain. This depends on the use of fowl pox vaccine as priming and inactivated AI-NDV vaccine as boosting of the immune system. Evaluation of the efficacy of such vaccination scheme is carried out in order to be applied in the control of both AI and NDV.

MATERIALS AND METHODS

A. Materials:

1. **Experimental birds:** A total of 150 one-day-old chicks were obtained from poultry production company, Egypt. They were floor reared, fed on a commercial Poultry ration and kept under good

hygienic conditions throughout the experiment.

2. **Antigen:**

A. **Challenge NDV strains:** Velogenic viscerotropic strain of NDV was obtained from Serum and Vaccine production. Institute, Abbasia, Egypt.

B. **Inactivated AI-NDV vaccine:** AI-ND KV Volvac. Cont. Neto/ Net Content: 500 ml/1000 ds, Inoculated at one day old.

3. **Candida albicans:** It was supplied by the Dept. of Mycology, Animal Health Research Institute. 24 hours old subculture of Candida albicans was used as antigen for evaluation of macrophages phagocytic activity.

4. **Media, reagents and chemicals:** RPMI 1640, Ficoll-hypaque, fetal calf serum, Giemsa strain and heparin preservative free (500 I-u/ ml) were obtained from Sermend Lab., Germany.

5. **Micrococcus lysodeikticus:** Sigma chemical Co., St.louis, USA.

6. **Griess reagent:** Sulphanimide, Naphthyl ethaylene diamine-di-hydrochloride, H3PO4.

B. **Methods:**

1. **Measurment of phagocytic activity of peripheral blood monocytes using candida albicans:** Separation of peripheral blood mononuclear cells using ficoll hypaque density gradient was carried out as described by Boyum (1968) .Mononuclear cell layer was collected, washed and resuspended in RPMI-1640 supplemented with 10% foetal calf serum and viability was done after Hanks and Wallace (1985). The test was performed according to procedure described by Anthony et al (1985) and Chu and Dietert (1989). Phagocytic percentage and index were estimated as follow:

$$\text{Phagocytic \%} = \frac{\text{No. of macrophages ingesting candida}}{\text{Total No. of Macrophages}} \times 100$$

$$\text{Phagocytic index} = \frac{\text{No. of macrophages ingesting more than 3 blastospores}}{\text{Total No. of macrophages with ingested blastospores}}$$

2. **Haemagglutination inhibition test (HI):** It was done as described by Beard (1989). It was used to evaluate humoral immune response.

3. **Lysozyme:** Lysozyme activity was measured by agarose gel Lysis assay, according to the method described by Schltz (1987). Briefly, lysoplates were prepared by dissolving 0.01% agarose in 0.06 MPBS at PH 6.3. 500 mg of uniform suspension of Micrococcus lysodeikticus in 5ml saline were added to 1 liter of agarose, plates were poured. Then, 25ul

of serum samples and standard lysozyme were added in each wells. After 18 hours the cleared zones diameter were measured to both standard lysozyme and serum sample and the concentration was estimated.

4. **Nitric oxide:** Determination of serum nitric oxide was carried out according to Green et al (1982) and Rajaraman et al (1998). Briefly 100ul of serum sample was transferred into flate-bottom 96-well ELISA plate and 100ul of Griess reagent were added to each well. The optical density was determined at 570nm with an ELISA plate reader. Absorbance of test samples was converted to 10um of nitrite by comparison with absorbance values of sodium nitrite standerd curve within linear curve fit.
5. **Experimental design:** One hundred and fifty one-day old commercial chicks were used in this study and were divided into 3 groups 50 chicks each:

Group (1): 50 chickens served as a control.

Group (2): chickens vaccinated with avian pox vaccine co-administered with inactivated AI- NDV at 10days.

Group (3): chickens vaccinated with inactivated AI-NDV vaccine at 10 days.

Two blood samples (a and b) were taken from 5 birds from each group at weekly intervals for 5 successive weeks via heart puncture.

a- Samples were taken in sterilized plastic centrifuge tube containing heparin for separation of mononuclear cells used in phagocytic activity.

b- Sample was taken without anticoagulant for serum separation and used for detection of antibody titer using (HI), lysozyme activity and Nitric oxide.

At the end of the experiment, 10 chickens from each group were challenged intramuscular with 0.2 ml suspension containing 10^6 virions of NDV velogenic strain (challenge test). Birds were kept under observation for 3 weeks with daily recording of symptoms and deathes.

Statistical analysis:

Data obtained were statistically analysed using analysis of variance and comparing between groups were performed using least significant difference (LSD) at $P< 0.05$ according to Petrie and Watson (1999) and computerized using SPSS (1999)

Table(1): Phagocytic % and index of chicken macrophages vaccinated with pox vaccine and/or vaccinated with AI-NDV vaccine

Group(s) /Days	15 days old		19 days old		26 days old	31 days old			38 days old	
	Phage %	Phage index	Phage %	Phage index	Phage %	Phage index	Phage %	Phage index	Phage %	Phage index
1	A 61±1.7	A 0.15±0.01	A 59±1.2	A 0.12±0.08	63±3.5	A 0.15±0.02	A 61±2.9	A 0.14±0.02	A 58±1.9	A 0.19±0.02
2	a 68±1.6	a 0.28±0.03	aB 70±2.0	aB 0.30±0.02	70±1.0	aB 0.33±0.01	a 71±2.2	a 0.32±0.04	aB 71±2.6	aB 0.34±0.02
3	65±2.2	a 0.23±0.05	b 61±1.7	ab 0.22±0.01	64±2.2	b 0.17±0.02	68±1.6	0.24±0.03	b 61±1.9	b 0.23±0.01

Aa,Bb significant different between two compared groups in the same column against capital letter at p <0.05 using least significant difference (LSD

Table(2): Serum lysozyme and nitric oxide of chicken vaccinated with pox vaccine and/or vaccinated with AI-NDV vaccine

Groups / days	15 days old		19 days old		26 days old		31 days old		38 days old	
	(ug/ml) lysozyme	Mm/ml Nitric oxide	(ug/ml) lysozyme	Mm/ml Nitric oxide	(ug/ml) lysozyme	Mm/ml Nitric oxide	(ug/ml) lysozyme	Mm/ml Nitric oxide	(ug/ml) lysozyme	Mm/ml Nitric oxide
1	10±2.2	27±1.6	A 16±5.6	25±1.8	A 49±10.0	31±2.3	40±5.0	30±2.5	A 37±5.0	34±3.7
2	14±6.0	23±1.4	a 37±5.5	23±4.3	a 96±14.0	24±2.0	50±12.0	36±6.9	a 61±7.0	30±3.4
3	14±2.2	24±2.8	24±4.3	23±4.5	72±11.0	30±4.0	58±10.0	34±2.4	43±6.0	31±3.5

Aa,Bb significant different between two compared groups in the same column against capital letter at p <0.05 using least significant difference (LSD)

Table(3)and Figure(1) Mean HI titer of NDV in chickens of different groups

Age Group(s)	HI Mean Titer Log(2)				
	1 day	7 days	15 days	21 days	28 days
1	3	2.5	0	0	0
2	3	3	2.5	4	4.25
3	3	2.8	2.3	2.5	2.6

- **Group 1 :** control non-vaccinated.
- **Group 2 :** vaccinated with avian-pox co-administered with AI-NDV inactivated vaccine.
- **Group 3 :** vaccinated with inactivated AI-NDV vaccine.

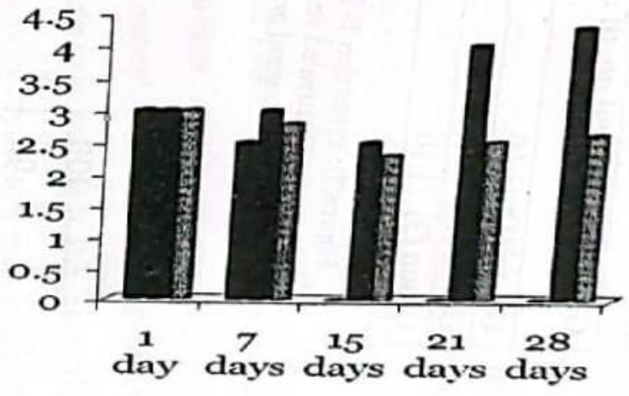
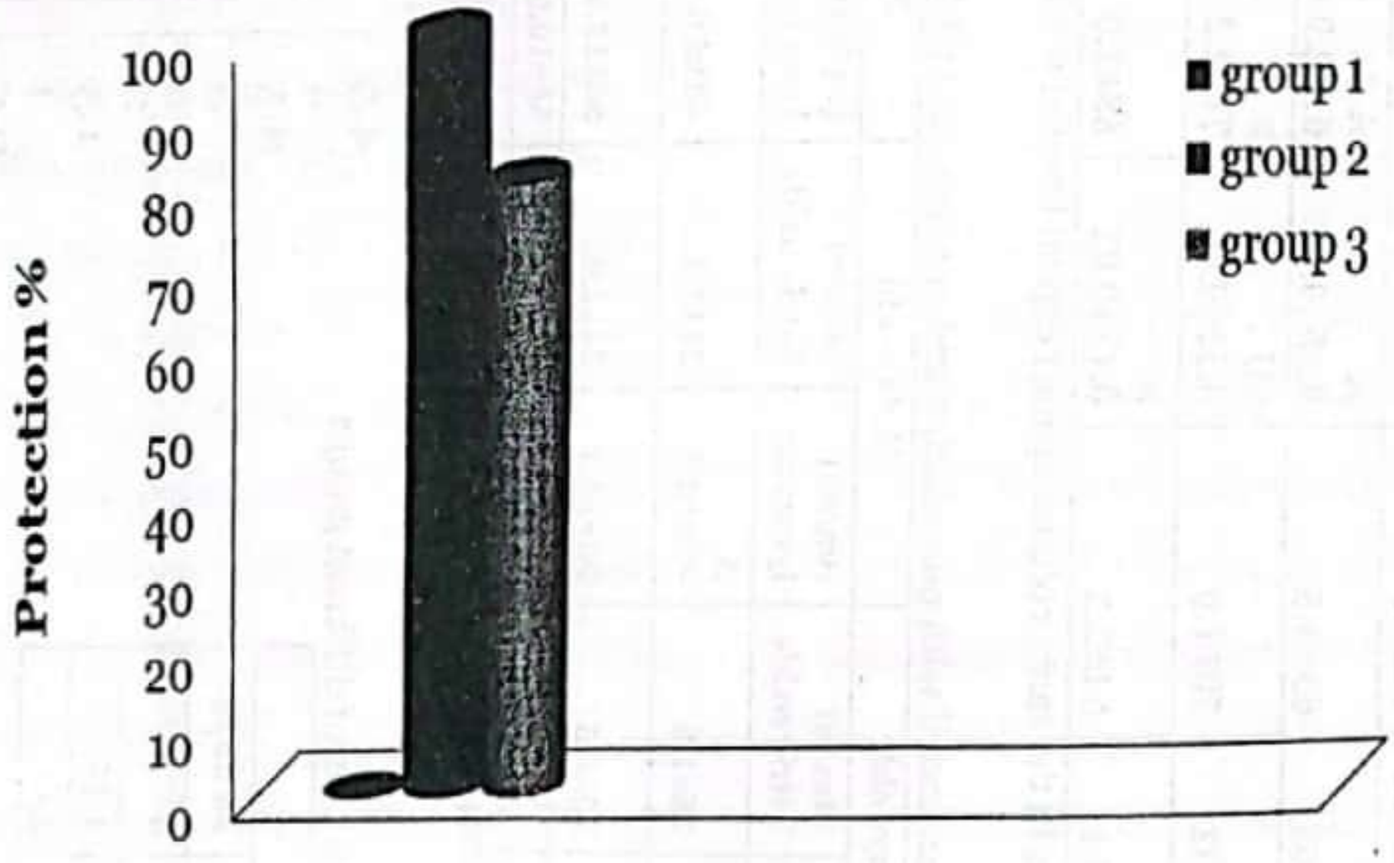


Table (4): protection rate of different groups of chickens against challenge with NDV (strain vvNDV74):

Group No.	Total no. of birds	Challenge test at 35 days of age		
		No.of dead birds	No. of survival	Protection %
Group (1)	10	10	0	0
Group (2)	10	0	10	100
Group (3)	10	2	8	80

Figure(2): Protection % induced by co-administration of avian pox vaccine with AI-NDV inactivated vaccine compared to administration of AI-NDV inactivated vaccine and control group



DISCUSSION

In the present study, chickens of group 2 revealed significant increase in phagocytic % and phagocytic index when compared to control group1 or compared to group 3.

Macrophages are known to play an important role in resistance to infection. They are part of non specific first line of defense because of their ability to engulf and degrade invading microorganism also secrete many different proteins such as lysozymal enzymes and cytokines that play a key role in regulating immunity (Tizard, 1996 and Stafford et al., 2002)

Our results agree with Ignatius et al., (2000) who noted that pox viruses belong to Avipox virus have been shown to activate human denretic cells and murine dendritic cells (Brown et al., 2000). Also, Drillien et al., (2004) demonstrated that modified vaccinia virus Ankara activate monocyte- derived human dendretic cells (DCs) as testified by an increase in surface co-stimulatory molecules and the secretion of pro-inflammatory cytokines. Moreover, Delaloye et al., (2009) reported the innate immune response was elicited by attenuated pox virus in human macrophages were characterized by robust chemokine production and fairly weak pro-inflammatory cytokine response. Similar results are obtained by Baxi and Oberio (1999) who found that chickens vaccinated with fowl pox vaccine had significant increase in T-lymphocyte count at 21 days post vaccination and 7 days post challenge.

Zhu et al., (2007) reported that vaccinia virus elicited innate immune response through both Toll-like receptor (TLR)- dependent and independent pathway, leading to the production of proinflammatory cytokines and interferon Beta that play together in achieving efficient activation of host defense. Indeed, our results and others demonstrate that pox vaccine enhance macrophage activity which subsequently reflect on specific immune response.

Lysozymes are proteins of low molecular weight found in polymorph nuclear cells and synthesized also in mononuclear cells. They are present in most tissue fluid except cerebrospinal fluid, sweat and urine. Lysozymes are considered as a member of innate humoral factors that elaborated from body and showed domestic increase in their concentration (Weir, 1983). Our results showed significant increase in chicken vaccinated with pox vaccine and inactivated AI-ND vaccine compared to chicken vaccinated only with inactivated AI-ND vaccine. There is no

available information concerning to the effect of pox vaccine on lysozyme activity. It is likely that the increase of lysozyme activity may be attributed to the activation of macrophage, the robust increase of chemokins production. There is no any significant change of nitric oxide among groups. Nitric oxide is a chemical messenger which has been recognized as important effectors molecules for macrophages in their cytostatic activity in fighting against invading pathogens and tumor cell target (Liew, 1995). Concerning to nitric oxide level in the serum, there is no significant difference between groups through the experimental period. However antibody titers of NDV vaccine were high in chicken of group 2 compared to group 3. On the level of protection against velogenic Newcastle disease virus, chickens vaccinated with pox vaccine co-administered AI –NDV (group2) showed the highest protection 100% while chicken vaccinated with inactivated AI-ND vaccine (group3) showed 80% ,our results are coincide with those obtained by Taylor et al., (1996) who demonstrated that inoculation of single dose of the recombinant fowl pox expressing the fusion and Hemagglutinin neuraminidase glycoprotein from velogenic NDV in chickens led to induction of significant increase of HI antibody titers that were maintained to 8 weeks .Further,it induced protective immunity against both lethal intramuscular NDV challenge and fowl pox virus challenge. It is speculate that, the high protection rate and high level of HI antibody titers in chiken received pox vaccine (group2) may be due to highly activated macrophage that produce potent amount of cytokine. This opinion confirmed by Delaloye et al., (2009) who found that modified vaccinia Ankara virus is a potent activator of IL-1B release by macrophages. IL-1B and IL-18 are key mediators of the host antimicrobial defense.

CONCLUSION

The multidirectional fashion of immune response with significant effect on antibodies in boosting response has been recorded. This is through induction of the immune modulatory protiens with differential synergy of complex-antigen specific effect. S/C injection of fowl pox vaccine at the age of 10 days specially before AI-NDV vaccination maximize the immune response of vaccinated birds and induce 100% protection when challenged at 4 weeks post vaccination with VVNDV to assess the protection. Results showed that the proposed vaccination scheme provide innate, humoral and cellular immunity. Also, it overcomes the effect of interference of maternal antibodies on the inactivated vaccines.

The study is the first to report the evaluation of prime and boost scheme of in commercial broiler chicken.

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نمط متعدد الاتجاهات من الاستجابة المناعية المستحدثة عن لقاح جدري الدجاج مشاركة مع لقاح النيوكاسل و الإنفلونزا الغير نشط

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*** قسم الدواجن طب بيطري جامعة المنوفية مدينة السادات

في هذه الدراسة تم تقييم التأثير التنشيطي للقاح جدري الطيور على الاستجابة المناعية السائلة والخلوية للقاح النيوكاسل و الإنفلونزا الغير نشط.

تم تقسيم دجاج التسمين إلى ثلاث مجموعات

المجموعة الأولى: مجموعة ضابطة للتجربة

المجموعة الثانية: تم تحصينها بلقاح النيوكاسل والإنفلونزا الغير نشط ولقاح جدري الدجاج تحت الجلد عند عمر 10 أيام

المجموعة الثالثة: تم تحصينها بلقاح النيوكاسل والإنفلونزا الغير نشط عند عمر 10 أيام

أجري اختبار التحدي للنيوكاسل بعد 4 أسابيع من التحصين كتقييم لاختبار الحماية، وتم تقييم المناعة السائلة والخلوية أسبوعياً وشملت مستوى أكسيد النيتريك ونشاط الليسوزيم، وقياس الأجسام المناعية HI للنيوكاسل، وكفاءة خلايا الماكروفاج الابتلاعية (phagocytosis)، وأظهرت النتائج نسبة حماية 100% ضد مرض النيوكاسل وزيادة معنوية في قدرة خلايا الماكروفاج وارتفاع في نسبة الليسوزيم والأجسام المناعية في الدجاج المحصن بالجدري ولقاح النيوكاسل والإنفلونزا- ونسبة حماية 80% في المجموعة المحصنة بالنيوكاسل والإنفلونزا فقط وصفر في المائة للمجموعة الضابطة.

كما أوضحت النتائج التأثير النشاطي المناعي للقاح جدري الدجاج بشكل أولي لتعزيز الاستجابة المناعية للقاح النيوكاسل والانفلونزا.